Functional analyses of FarA transcription factor involved in biodegradable plastic degradation and fatty acid metabolismin Aspergillus oryzae

	シャロン　マリー　バヘナ　ガリド
氏名（本籍地）	SHARON MARIE BAHENA GARRIDO
学位の種類	博士（農学）
学位記番号	農博第 1060 号
学位授与年月日	平成 25 年 3 月 27 日
学位授与の要件	学位規則第 4 条第 1 項
研究科，専攻	東北大学大学院（博士課程）農学研究科生物産業創成科学専攻
論文題目	Functional analyses of FarA transcription factor involved in biodegradable plastic degradation and fatty acid metabolismin Aspergillus oryzae （麹菌の生分解性プラスチック分解酵素生産と脂肪酸代謝に関与する転写因子ＦａｒＡの機能解析）
博士論文審査委員	（主査）教授　五味勝也 教授　阿部敬悦 教授　米山　裕

論 文 内 容 要 旨

Introduction

Aspergillus oryzae has been extensively used as *koji* (solid-state culture of *koji*-mold) in the manufacture of traditional Japanese fermented products, such as sake (rice wine), shoyu (soy sauce), and miso (soybean paste) for more than 1,000 years (Hara et al. 1992; Machida 2002; Machida et al. 2008; Yu et al. 2005). The most important role of *koji* is to supply various useful enzymes produced by *A. oryzae*, for example, amylases, proteases and lipases. Recently, among these lipolytic enzymes particularly cutinase, CutL1, produced by *A. oryzae* has been found to degrade biodegradable plastic, poly-(butylene succinate-*co*-adipate) (PBSA) (Maeda et al. 2005). PBSA is produced through the copolymerizaion of a glycol, such as 1,4-butanediol, with an aliphatic dicarboxylic acid, such as succinate (Fujimaki 1998) with a small amount of adipate. On the other hand, cutinases are well known enzymes produced by phytopathogenic and insect-pathogenic fungi (Purdy and Kolattukudy 1975; Sweigard et al. 1992) and catalyze the hydrolysis of ester bonds from fatty acid polymers, thus facilitating fungal penetration through the cuticle that covers over the cell surface (Kolattukudy 1985; Voigt et al. 2005).

Regulation of fungal cutinase genes has been extensively investigated in the plant pathogenic fungus, *Fusarium solani* f. sp. *pisi* (teleomorph, *Nectria haematococca*), which has three cutinase genes, namely *cut1*, *cut2*, and *cut3*. The amino acid sequences of these cutinases share a high degree identity to each other. It has been reported that expression of *cut1* is strongly induced by cutin monomers while *cut2* and *cut3* are expressed constitutively at basal level (Li et al. 2002). Induction of the *cut1* gene expression by cutin monomers is mediated by the transcriptional factor CTF1α (Li and Kolattukudy 1997; Li et al. 2002). This zinc finger transcriptional factor CTF1α in *F. solani* has ~70% similarity with FarA in *Aspergillus nidulans*, also a $Zn(II)_2Cys_6$ transcription factor which also upregulates genes required for growth on fatty acids (Hynes et al. 2006). In addition, the orthologs of *F. solani* CTF1α and *A. nidulans* FarA have been recently described in another plant pathogen *Fusarium oxysporum*, human pathogenic yeast *Candida albicans*, and alkane assimilating yeast *Yarrowia lipolytica*, and these orthologs are involved in fatty acid utilization (Martínez-Rocha et al. 2008; Poopanitpan et al. 2010; Ramírez and Lorenz 2009). In *Aspergilli*, it has been reported that FarA is involved in fatty acid metabolism (Hynes et al. 2006), but there is no information on the involvement of this transcription factor in the production of lipolytic enzymes including cutinases. In this study, we report that FarA transcriptional factor also

works in the regulation of genes responsible for the production of lipolytic enzymes such as cutinase as well as of hydrophobic proteins for the degradation of a biodegradable plastic, PBSA, in *A. oryzae.* In addition, it discusses the regulation of genes for fatty acid metabolism and glyoxylate cycle as that of the *A. nidulans*; and the pleiotropic effects of *farA* disruption through microarray analysis that have never been demonstrated before across other filamentous fungal species.

Chapter 1

Involvement of FarA transcription factor in the degradation of biodegradable plastic in *Aspergillus oryzae*

Construction of the *farA* disruptants were performed (Figure 1) and homokaryotic disruptants were subjected to assay for growth phenotype on the agar minimal medium containing PBSA as a sole carbon source. As shown in Figure 2, wild-type (WT) strain could grow and formed halos around the mycelial colonies. The *farA* disruptants, on the other hand, showed a restricted growth and did not form halo around the colonies. This indicated that the wild-type produced lipolytic enzymes such as cutinase, CutL1 (Maeda et al. 2005), to degrade PBSA in the agar medium, while the disruptants did not.

It was previously reported that CutL1 was produced in the presence of biodegradable plastic, PBSA by *A. oryzae* and was able to degrade PBSA efficiently (Maeda et al. 2005). Production of *F. solani* cutinase homologous to CutL1 has shown to be regulated by the Zn_2Cys_6 transcription activator CTF1α. Since there exists a CTF1α ortholog (FarA) in the *A. oryzae* genome, it was expected that the FarA transcription factor could also regulate the *cutL1* gene expression in *A. oryzae*. In fact, the *farA* disruption resulted in loss of degradation activity toward PBSA accompanied with the absence of CutL1 protein in the agar medium Figure 3A) and in a significant decrease in the *cutL1* gene expression (Figure 4A), indicating that also in *A. oryzae* the FarA transcription factor is required for cutinase (CutL1) production. Table 2 shows the lipase activities of the wild-type and the *farA* disruptant strains.

For further efficient PBSA degradation, hydrophobic surface binding proteins such as HsbA and hydrophobin RolA contribute to recruit cutinase proteins onto the PBSA surface and consequently facilitated PBSA degradation (Takahashi et al. 2005; Ohtaki et al. 2006). In this regard, interestingly, the *farA* disruption resulted in a reduced expression of the *hsbA* gene (Figure 4A) and in the disappearance of the HsbA protein (Figure 3C). The result

suggested that the HsbA protein was induced by the FarA transcription factor as well as CutL1 for degradation of PBSA. This could be supported by the presence of the consensus sequence of *cis*-element 5′-CCTCGG-3′ for *A. nidulans* FarA binding in the promoter regions of the *cutL1* and *hsbA* genes. In contrast, the expression level of the *rolA* gene (Figure 4A) was not significantly affected by the *farA* disruption, which was predicted from the absence of the consensus *cis*-element in the *rolA* promoter.

Furthermore, under the submerged culture condition with oleic acid as a sole carbon source, WT strains expressed significantly higher transcript levels of genes for extracellular lipases, such as *mdlB* and *tglA*, compared to the *farA* disruptants (Figure 4B). Extracellular lipases generally catalyze the hydrolysis of triglycerides to fatty acids, diacylglycerol, monoacylglycerol, and glycerol at an oil-water interface and are extensively used for commercial industries. On the industrial point of view, it would be worthwhile to note that the expression of the lipase genes in *A. oryzae* was affected by the disruption of the *farA* gene, which opens further research works such as overexpression of FarA in *A. oryzae* to produce higher amount of these lipases and its optimization for industrial applications. In addition, it would be expected that *farA* overexpression leads to simultaneous hyperproduction of cutinase CutL1 and hydrophobic surface binding protein HsbA, resulting in further efficient degradation of biodegradable plastic such as PBSA by *A. oryzae*.

Chapter 2

Involvement of FarA and other Zn finger motif transcription factors for fatty acid metabolism in *Aspergillus oryzae*

Phenotypes of the *farA* disruptant grown on short and long-chain fatty acids as sole carbon sources were also considered in the study (Figure 5). As reported by Hynes et al (2006), in *A. nidulans*, FarA is involved in fatty acid metabolism and its disruption results in loss of the assimilation of both short- and long-chain fatty acids. Interestingly, however, we found that the *farA* disruption only slightly affected growth on the short- and long chain fatty acids as sole carbon sources and the disruptant exhibited a modest growth nearly indistinguishable from the WT, inconsistent with the growth phenotype of the *A. nidulans* counterpart (in which restricted growth was observed or loss of the ability to utilize fatty acids). However, genes for fatty acid metabolism contain the consensus sequence of *cis*-element 5′-CCTCGG-3′ for *A. nidulans* FarA binding in their promoter regions. Most of these

genes (i.e. fatty-acyl CoA oxidase, carnitine O-acyltransferase, malate synthase) were downregulated in the *farA* disruptant as compared to the WT when induced with oleic acid (Figure 8). Inconsistencies in the phenotypes and the gene expressions of the *farA* disruptants were evident in the study. Hence, we further hypothesized that fatty acid assimilation pathway may be regulated by not only FarA but another so-far-unidentified transcription factor(s) in *A. oryzae*. Such a candidate transcription factor would be *A. nidulans* FarB that participates in the regulation of genes for fatty acid metabolism (Hynes et al, 2006). The *farB* gene has been identified in *A. nidulans* and disruption of *farB* abolishes growth on short-chain fatty acids but not on long-chain fatty acids. In addition, although FarB has a typical Zn_2Cys_6 binuclear motif that is not highly homologous to that of FarA, electrophoretic mobility shift assays revealed that FarB could also bind to the consensus *cis*-element of 5′-CCTCGG-3′ (Hynes et al, 2006). An ortholog of *A. nidulans* FarB (AO090103000027) was also found in the *A. oryzae* genome, and it exhibited a high homology of 81% at amino acid level with *A. nidulans* FarB. Unfortunately, we have not yet been able to obtain a homokaryotic *farB* disruptant and were ultimately unsuccessful to construct double mutants of these two transcriptional factors for further analysis. This technical difficulty of obtaining a *farB* disruptant ignites a question whether this FarB transcriptional factor is essential in the growth of *A. oryzae* and that would be another interesting work in the future. However, in this study the constructed heterokaryotic *farB* disruptant strains were still considered and grown in fatty acid sources (Figure 6). The growth was inhibited in the hexoanoate and laurate, both are medium-chain fatty acids. The study proceeded further to include the possible involvement of other Zn finger motif genes in response to short-, medium- and long-chain fatty acid metabolism in *A.oryzae*. *facB* gene and other unidentified Zn finger motif genes were being studied and analyzed in the study in which the transcription factor disruptant library from the NODA Research Institute was being utilized. An ortholog of *A. nidulans* FacB was also found in the *A. oryzae* genome and it also exhibited a high homology of 81% at amino acid level with *A. nidulans* FacB. *A. nidulans* FacB is responsible for the acetate metabolism but it is hypothesized to take part in the fatty acid metabolism. Expressions of genes for tricarboxylic acid cycle or glyoxylate cycle were analyzed in the *facB* disruptant and compared to the WT. Most of these genes (i.e. malate synthase, methylcitrate synthase) were downregulated in the *facB* disruptant when induced with acetate, propionate, and butyrate (Figure 9). This suggests that *facB* gene is responsible for the short-chain fatty acid metabolism and the regulation of genes for glyoxylate cycle. From the above results, the regulation of genes for fatty acids in *A.oryzae* could be accounted to the roles of

FacB (short-chain fatty acids), FarA and FarB (medium-chain fatty acids), and another so-far-unidentified transcription factors for the long- chain and complex fatty acids metabolism.

Chapter 3

Pleiotropic effects of *farA* disruption in *Aspergillus oryzae*

As discussed earlier, FarA is already known to regulate cutinase genes in *Fusarium solani* and *A. oryzae* (Li et al. 1997; Li et al. 2002; Garrido et al. 2012). However, it is further believed that FarA may regulate or affect other genes which have not been reported yet, including those regulated by pleiotropic effects. Microarray analysis was conducted between the wild-type (WT) and the *farA* disruptant induced with oleic acid. From the microarray analysis data between the wild-type (WT) and the *farA* disruptant induced with oleic acid, results confirmed by qRT-PCR showed that interestingly, a number of genes encoding ribosomal proteins such as 60S ribosomal proteins L10, L18 and L19 (data not shown), and 40S ribosomal proteins S16 and 40S ribosomal protein/ubiquitin (Figure 10) were down-regulated in the *farA* disruptant as compared to the WT. Each gene contains the *cis*-element 5′ -CCTCGG-3′ (complement, 5′-CCGAGG-3′) of FarA transcriptional factor in their 5′ upstream regions. These ribosomal gene expressions were also determined under different fatty acid sources such as the palmitic acid and Tween 20, and results showed that genes were also downregulated in the *farA* disruptant as compared to the WT. Furthermore, *farA* complement strain was also constructed and its gene expressions were determined. These ribosomal gene expressions were rescued/revived/restored in the *farA* complementation suggesting that these ribosomal gene expressions during fatty acid metabolism may be FarA dependent. However, it would be relevant to note also that from same microarray data, there were a number of ribosomal genes that were still affected by *farA* disruption even in the absence of its *cis*-element in their promoter regions and other genes may seem not affected by *farA* disruption itself and that it suggests that *farA* disruption may indirectly affect some of the ribosomal genes or that other transcription factors (their interactions/coordination) may play in the regulation of ribosomal biogenesis in general. Ribosomal biogenesis is both necessary for cellular adaptation, growth and proliferation as well as a major energetic and biosynthetic demand upon cells (Leary and Huang, 2001). However, little is known about the mechanisms and genes involved in ribosomal biogenesis in eukaryotes especially among *Aspergilli* species. The probable role of FarA transcription factor in the regulation of a number of ribosomal genes represents only a small fraction of a larger picture of ribosomal

gene regulation in filamentous fungi.

In summary, the *farA* disruptants were unable to degrade PBSA efficiently because of loss of the production of CutL1 and HsbA, resulted from the significantly lower expression levels of those encoding genes in the disruptants. Furthermore, expressions of the extracellular lipase genes such as *mdlB* and *tglA* were also significantly decreased by the *farA* disruption. These results may unravel the role of FarA transcription factor in the regulation of cutinase and lipase genes and in the productions of lipolytic enzymes in *A. oryzae*. However, growth phenotypes on short- and long-chain fatty acids of *A. oryzae farA* disruptants were inconsistent with those of *A. nidulans* counterparts. We further concluded that the fatty acid assimilation pathway may be regulated by not only FarA but another so-far-unidentified transcription factor(s) in *A. oryzae*. From the study we identified FacB transcription factor may regulate genes for metabolism of short-chain fatty acids, FarA for the medium-chain fatty acids and one candidate Zn finger motif gene for the metabolism of long- and complex fatty acids. Furthermore, a number of genes encoding ribosomal proteins such as 60S ribosomal proteins L10, L18 and L19, and 40S ribosomal proteins S16 and 40S ribosomal protein/ubiquitin were affected by *farA* disruption. The probable role of FarA transcription factor in the regulation of a number of ribosomal genes represents only a small fraction of a larger picture of ribosomal gene regulation in filamentous fungi. Further studies are required to determine the initial signals, signal transduction pathways utilized, and how these are used to control ribosome biogenesis as a whole.

References:

Fujimaki T (1998) Processability and properties of aliphatic polyesters, 'BIONOLLE', synthesized by polycondensation reaction. Polym Degrad Stabil 59:209–214.

Hara S, Kitamoto K, Gomi K (1992) New developments in fermented beverages and foods with *Aspergillus*. pp. 133–153. *In* J. W. Bennett and M. Klick (ed.), *Aspergillus*: Biology and Industrial Applications (Biotechnology Series, Vol. 23), Butterworth-Heinemann, Oxford.

Hynes MJ, Murray SL, Duncan A, Khew GS, Davis MA (2006). Regulatory genes controlling fatty acid catabolism and peroxisomal functions in the filamentous fungus *Aspergillus nidulans*. Eukaryot Cell 5: 794–805.

Kolattukudy PE, Rogers LM, Li D, Hwang CS, Flaishman MA (1995) Surface signaling in pathogenesis. Proc Natl Acad Sci USA 92:4080–4087

Li D, Kolattukudy PE (1997) Cloning of cutinase transcription factor 1, a transactivating protein containing Cys_6Zn_2 binuclear cluster DNA binding motif. J Biol Chem 272: 12462–12467.

Li D, Sirakova T, Rogers L, Ettinger W, Kolattukudy PE (2002) Regulation of constitutively expressed and induced cutinase genes by different zinc finger transcription factors in *Fusarium solani* f. sp. *pisi* (*Nectria haematococca*). J Biol Chem 277: 7905–7912.

Machida M (2002) Progress of *Aspergillus oryzae* genomics. Adv Appl Microbiol 51:81–106

Machida M, Yamada O, Gomi K (2008) Genomics of *Aspergillus oryzae*: learning from the history of Koji mold and exploration of its future. DNA Res 15:173–183.

Maeda H, Yamagata Y, Abe K, Hasegawa F, Machida M, Ishioka R, *et al.* (2005) Purification and characterization of a biodegradable plastic-degrading enzyme from *Aspergillus oryzae*. Appl Microbiol Biotechnol 67: 778–788.

Martínez-Rocha, A. L., Di Pietro, A., Ruiz-Roldán, C., and Roncero, M. I.: Ctf1, a transcriptional activator of cutinase and lipase genes in *Fusarium oxysporum* is dispensable for virulence, Mol. Plant Pathol., 9, 293–304 (2008).

Ohtaki, S., Maeda, H., Takahashi, T., Yamagata, Y., Hasegawa, H., Gomi, K., Nakajima, T., and Abe, K.: Novel hydrophobic surface binding protein, HsbA, produced by *Aspergillus oryzae*, Appl. Environ. Microbiol., 72, 2407–2413 (2006).

Poopanitpan, N., Kobayashi, S., Fukuda, R., Horiuchi, H., and Ohta, A.: An ortholog of farA of Aspergillus nidulans is implicated in the transcriptional activation of genes involved in fatty acid utilization in the yeast *Yarrowia lipolytica*, Biochem. Biophys. Res. Commun., 402, 731–735 (2010).

Purdy RE, Kolattukudy PE (1975) Hydrolysis of plant cuticle by plant pathogens. Properties of cutinase I, cutinase II, and a nonspecific esterase isolated from *Fusarium solani* pisi. Biochemistry 14:2832–2840.

Ramírez, M. A. and Lorenz, M. C.: The transcription factor homolog CTF1 regulates **β**-oxidation in *Candida albicans*, Eukaryot. Cell, 8, 1604–1614 (2009).

Sweigard JA, Chumley FG, Valent B (1992) Cloning and analysis of CUT1, a cutinase gene from *Magnaporthe grisea*. Mol Gen Genet 232:174–182.

Takahashi, T., Maeda, H., Yoneda, S., Ohtaki, S., Yamagata, Y., Hasegawa, F., Gomi, K., Nakajima, T., and Abe, K.: The fungal hydrophobin RolA recruits polyesterase and laterally moves on hydrophobic surfaces, Mol. Microbiol., 57, 1780–1796 (2005).

Voigt CA, Schäfer W, Salomon S (2005) A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J 42: 364–375.

Yu J, Proctor RH, Brown DW, Abe K, Gomi K, Machida M, Hasegawa F, Nierman WC, Bhatnagar D, Cleveland TE (2004). Genomics of economically significant *Aspergillus* and *Fusarium* species. In: Arora KD, Khachatourians GG (eds) Applied Mycology and Biotechnology, vol 4. Fungal genomics. Elsevier, Amsterdam.

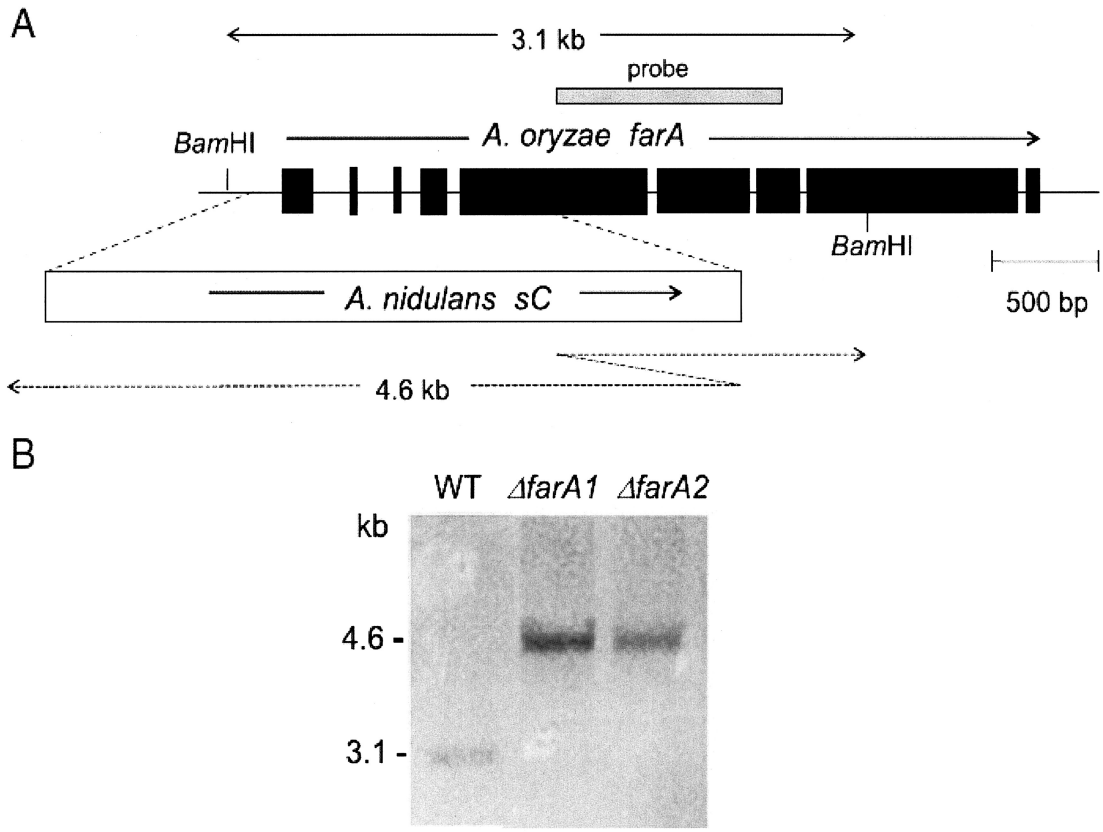

Fig. 1. Structure of *A. oryzae farA* gene and Southern blot analysis of the disruptants. (A) Structure of the *farA* gene and the gene disruption strategy. Exons of the *farA* gene are shown by black boxes. Each probe used for hybridization was obtained by PCR with primer sets, *farA*probeF and *farA*probeR (Table 1). *Bam*HI restriction enzyme sites used for Southern blot analysis of the disruptants are also shown. WT, wild-type; *ΔfarA1* and *ΔfarA2*, independent clones obtained by *farA* disruption.

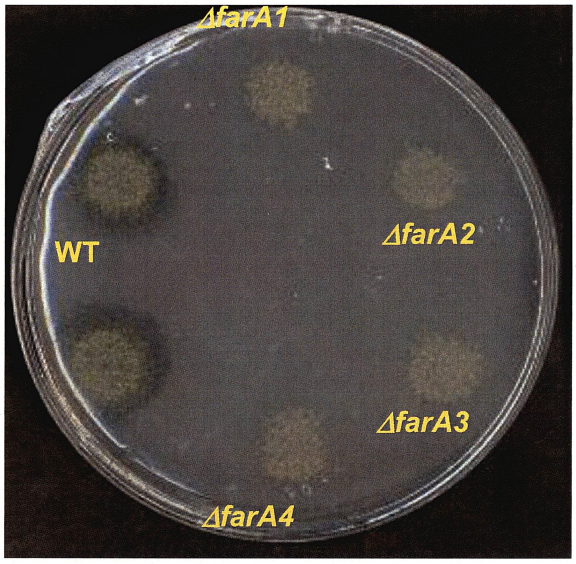

Fig. 2. Growth and halo formation of the wild-type and the disruptant strains grown on agar minimal medium with 0.4% PBSA as a sole carbon source. Approximately 100 conidiospores of each strain was spot inoculated and grown for 5 d at 30°C. Note that clear haloes are observed around the colonies of the wild-type (WT), while no halo formation of the *farA* disruptants (*ΔfarA1–ΔfarA4*).

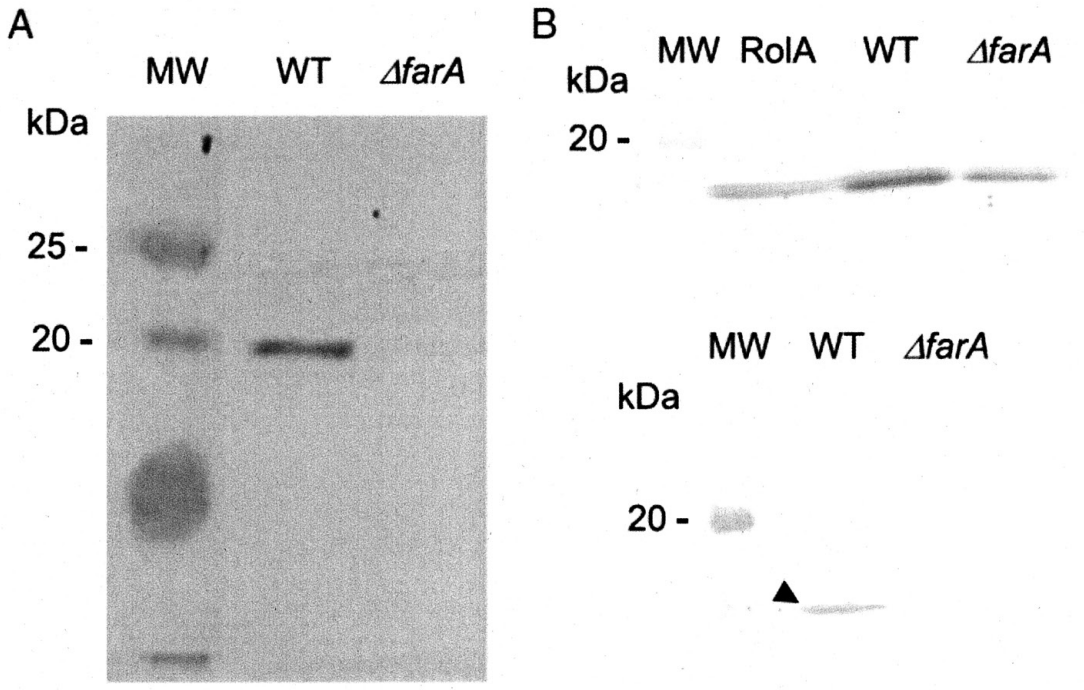

Fig. 3. Western blot analysis of the wild-type (WT) and the *farA* disruptant strains. Haloes and the surrounding agar of the colonies were recovered and shaken for 3 h with phosphate buffer at 30°C. The resultant supernatants were precipitated with trichloroacetic acid, followed by resolving in phosphate buffer. Proteins (0.5 mg and 0.3 mg for the wild-type and disruptant, respectively) were applied onto SDS-PAGE for Western blotting for the presence of CutL1, RolA and HsbA proteins. (A) Western blot analysis of the CutL1 protein produced by the wild-type (WT) and the *farA* disruptant (*ΔfarA*). (B) Western blot analysis of the RolA protein produced by WT and *ΔfarA*. RolA, authentic RolA protein applied. (C) Western blot analysis of the HsbA protein produced by WT and *ΔfarA*.

Table 2 Lipase activities of the wild-type and the *farA* disruptant strains.

	Lipase activity (U/mg of protein)	
	p-Nitrophenyl butyrate	**p-Nitrophenyl palmitate**
WT	**1.01±0.07**	**0.53±0.03**
***farA* disruptant**	**0.65±0.04**	**0.14±0.02**

Haloes and the surrounding agar of the colonies were recovered and shaken for 3 h with phosphate buffer at 30°C. The resultant supernatants were analyzed for assay of lipase activity. Production of **p**-nitrophenol in both assays was measured at 410 nm. The values are means of three independent experiments with standard deviations. One unit of activity was defined as the amount of enzyme required to release 1 **μ**mol pNP per min from pNPB or pNPP under standard reaction condition (37°C, pH 8.0).

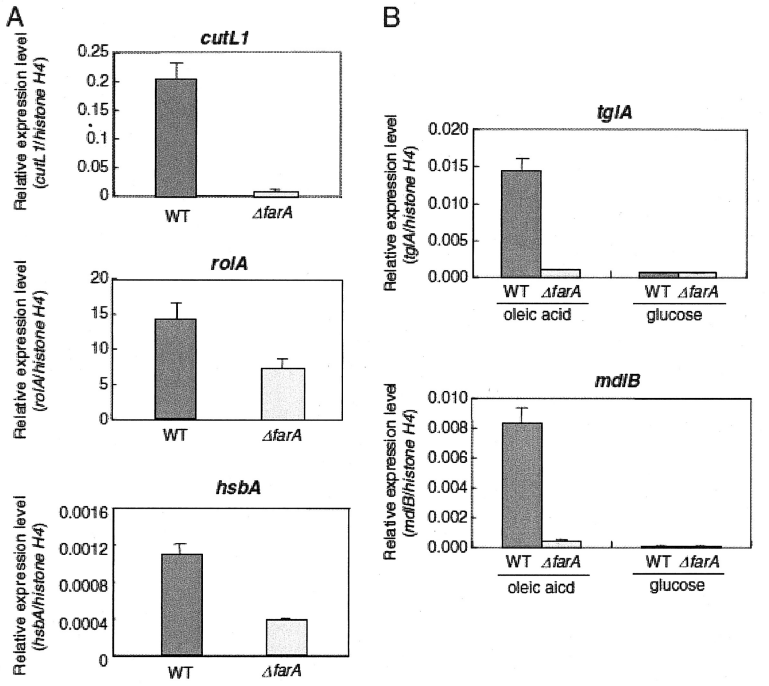

Fig. 4. Gene expression analyses of the lipolytic genes and hydrophobic surface binding protein genes by qRT-PCR. (A) The wild-type (WT) and the *farA* disruptant (*ΔfarA*) were grown on agar minimal medium containing 0.4% PBSA and incubated for 5 days at 30°C. Mycelia were taken out, ground for total RNA preparation in liquid nitrogen and used for gene expression analysis. (B) WT and *ΔfarA* grown in liquid CD medium with 2% fructose for 36 h at 30°C. Mycelia then were collected and transferred to the minimal medium with 1% oleic acid or 1% glucose as the sole carbon source. The relative expression level was normalized to that for the histone H4 gene. The values are means of three independent experiments, and the error bars denote standard errors.

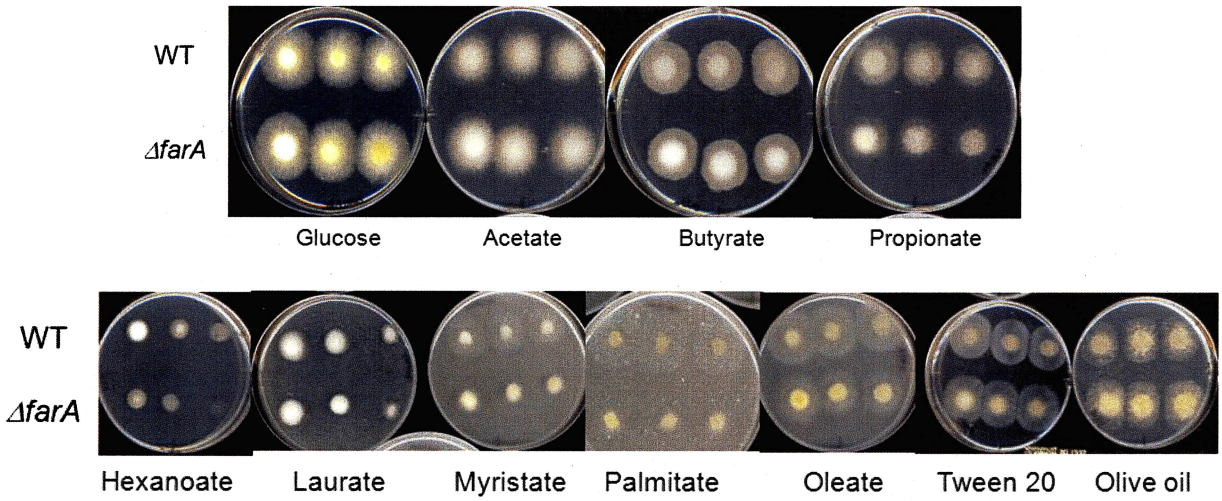

Fig. 5. Growth of the wild-type and the *farA* disruptant strains on minimal medium with 0.3% of long-, medium- and short-chain fatty acids, namely acetate, butyrate, propionate, hexanoate, laurate, myristate, palmitate, oleate, Tween 20 and olive oil emulsified with 0.5% Tergitol (NP-40, Sigma-Aldrich) and added to the agar medium. On each agar plate the decreasing number of spores was inoculated from left to right. Growth of both wild-type and disruptant on 0.1% fatty acid medium was almost the same as that on 1% fatty acid medium (data not shown).

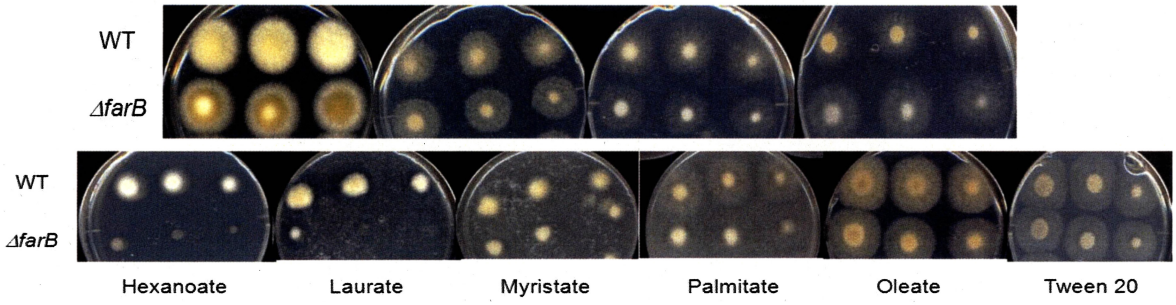

Fig. 6. Growth of the wild-type and the heterokaryotic *farB* strains on minimal medium with 0.3% of long, medium- and short-chain fatty acids, namely acetate, butyrate, propionate, hexanoate, laurate, oleate, myristate, palmitate, oleate, Tween 20 and olive oil emulsified with 0.5% Tergitol (NP-40, Sigma-Aldrich) and added to the agar medium. On each agar plate the decreasing number of spores was inoculated from left to right.

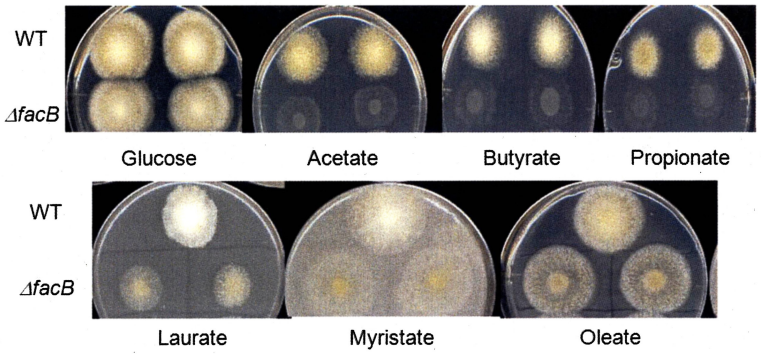

Fig.7. Growth of the wild-type and the *facB* strains on minimal medium with 0.3% of long-, medium- and short-chain fatty acids, namely acetate, butyrate, propionate, laurate, myristate, and oleate emulsified with 0.5% Tergitol (NP-40, Sigma-Aldrich) and added to the agar medium. On each agar plate the decreasing number of spores was inoculated from left to right.

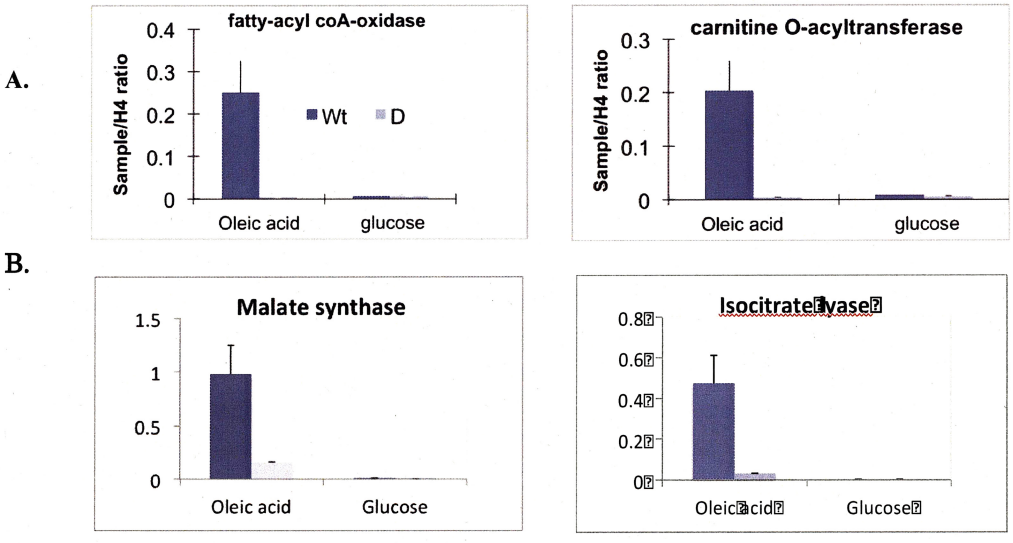

Figure 8. Expression analyses of genes for beta-oxidation (A) and the glyoxylate cycle (B) in the WT and *farA* disruptant by qRT-PCR. Samples were grown in liquid CD medium with 2% fructose for 36 h at 30°C. Mycelia then were collected and transferred to the minimal medium with 1% oleic acid, palmitic acid and Tween 20 and 1% glucose as the sole carbon source. The relative expression level was normalized to that for the histone H4 gene. The values are means of three independent experiments, and the error bars denote standard errors.

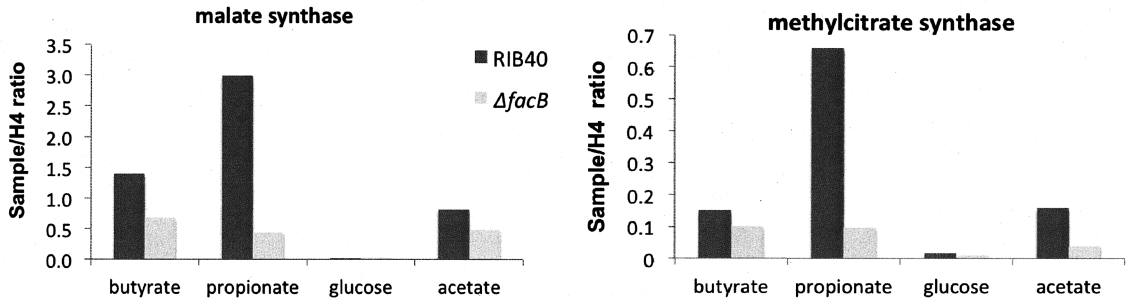

Fig. 9. Expression analyses of the genes for glyoxylate cycle by qRT-PCR. The wild-type (WT) and the *facB* disruptant (*ΔfacB*) were grown in liquid CD medium with 2% fructose for 36 h at 30°C. Mycelia then were collected and transferred to the minimal medium with 1% oleic acid or 1% glucose as the sole carbon source. The relative expression level was normalized to that for the histone H4 gene. The values are means of three independent experiments, and the error bars denote standard errors.

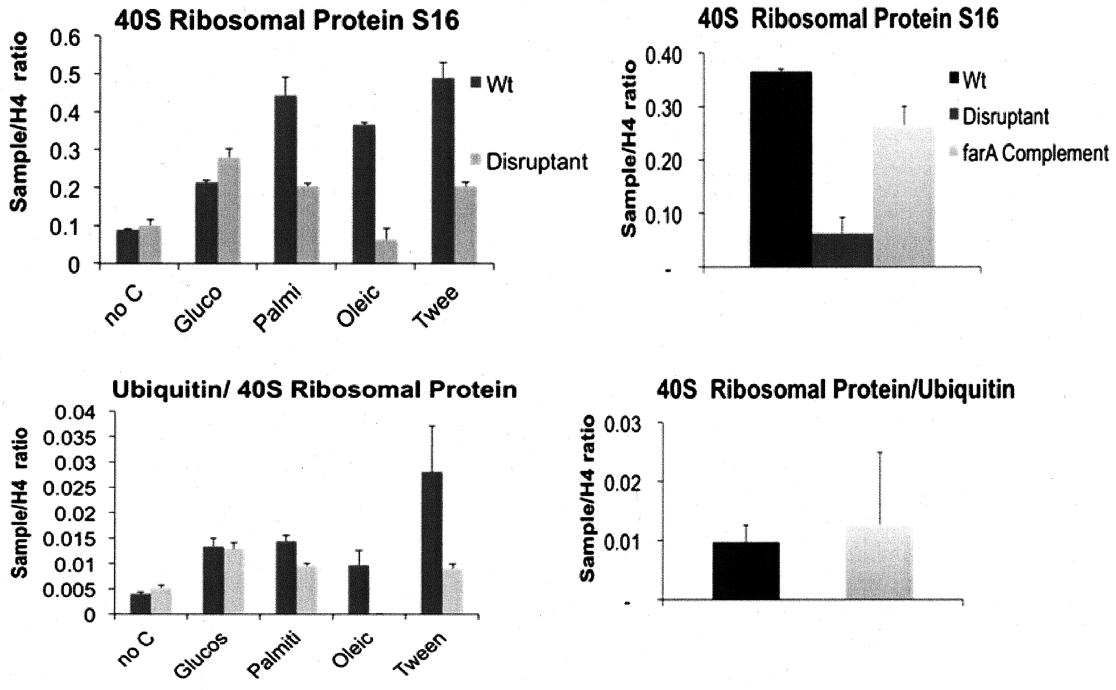

Fig. 10. Expression analyses of ribosomal genes by qRT-PCR. The wild-type (WT), the *farA* disruptant (D) and the *farA* complement were grown in liquid CD medium with 2% fructose for 36 h at 30°C. Mycelia then were collected and transferred to the minimal medium with 1% oleic acid, palmitic acid and Tween 20 and 1% glucose as the sole carbon source. The relative expression level was normalized to that for the histone H4 gene. The values are means of three independent experiments, and the error bars denote standard errors.

論文審査結果要旨

麹菌の生産するリパーゼの一種であるクチナーゼ(CutL1)は、生分解性プラスチックの一種であるポリブチレンスクシネートを分解する酵素として見出された。CutL1 は植物病原菌 *Fusarium solani* が生産するクチナーゼに相同性が高く、*F. solani* では転写因子 CTF1αがクチナーゼ生産を制御していることが報告されている。一方、麹菌の近縁種である *Aspergillus nidulans* においてCT F1αオーソログの FarA は脂肪酸代謝に関与していることが知られている。しかし、生分解性プラスチック分解酵素として単離された麹菌クチナーゼの生産に関わる転写因子については何ら知見がなかったことから、本研究では麹菌の FarA オーソログを対象として、その機能解析を行った。

麹菌ゲノムデータベースから FarA オーソログを探索し、その遺伝子破壊株を作製し、生分解性プラスチックを含む寒天培地上での生育を調べたところ、破壊株ではプラスチック分解に伴うクリアゾーンが認められなかった。コロニー周辺の寒天培地からタンパク質を抽出し、抗 CutL1 抗体を用いたウェスタン解析により、破壊株では CutL1 の生産が認められなかった。さらに定量 PCR の結果、破壊株では *cutL1* 遺伝子の発現がほとんど認められなかった。麹菌による生分解性プラスチック分解の際に生産されるハイドロフォビンなどの両親媒性タンパク質の遺伝子発現についても調べたところ、興味深いことに破壊株では *hsbA* 遺伝子の発現が顕著に低下していた一方で、*rolA* 遺伝子は半分程度の発現低下しか示さなかった。また、麹菌が分泌生産する 2 種類のリパーゼ TglA, MdlB の遺伝子も破壊株で著しい発現低下が認められた。このように、麹菌の FarA はリパーゼ遺伝子と両親媒性タンパク質 HsbA の遺伝子の発現に重要な転写因子であることが明らかとなった。一方、麹菌の脂肪酸代謝に対する影響を調べるため、各種脂肪酸培地の生育を調べたところ、*farA* 破壊株では中鎖脂肪酸の資化能が僅かに低下していたが、FarA に相同性を示す転写因子 FarB の変異株では *farA* 破壊株よりも中鎖脂肪酸の資化能の低下が認められた。これらの結果は *A. nidulans* における *farA/farB* 欠損の脂肪酸資化能に対する影響とは異なっており、麹菌と *A. nidulans* では FaA と FarB の脂肪酸代謝に関わる機能に違いがあることが示唆された。さらに、興味深いことに *farA* 破壊株ではリボソームタンパク質の遺伝子群の発現が抑制されており、FarA のリボソームタンパク質制御機能も示唆された。本研究では麹菌の転写因子 FarA のリパーゼと両親媒性タンパク質遺伝子の発現ならびに中鎖脂肪酸の資化への関与を明らかにし、*farA* 高発現による有用菌株の造成に有用な知見を得ることができた。以上のことから、審査員一同は本論文提出者に対し博士（農学）の学位を授与するに値するものと判定した。